SVI Recommendation for *De Novo* Criteria (PS2 & PM6) - Version 1.1

The Sequence Variant Interpretation (SVI) Working Group proposes a point-based system to determine the strength of *de novo* evidence (ACMG/AMP criteria codes PS2 and PM6) based upon three parameters:

- confirmed parental relationships versus assumed parental relationships status
- phenotypic consistency
- number of *de novo* observations

To determine the appropriate strength level to apply for *de novo* occurrence(s), each proband with a *de novo* variant is awarded a point value based upon phenotypic consistency and confirmed or assumed parental relationships (Table 1). The combined point value of all *de novo* occurrences is then compared to Table 2 to determine the applicable evidence strength level. For example, if a *NIPBL* variant was *de novo* in one patient with Cornelia de Lange syndrome, with confirmed parental relationships (2 points; Table 1) and *de novo* in two additional unrelated patients with Cornelia de Lange syndrome with unconfirmed parental relationships (1 + 1 points; Table 1), then VeryStrong evidence level is applied (PS2_VeryStrong) based on combined point value of 4 (Table 2). If the parents have not been tested for parentage or for the variant, no points should be awarded.

Table 1. Points* awarded per *de novo* occurrence

Phenotypic consistency	Points per Proband	
	***de novo* with confirmed parental relationships**	***de novo* with unconfirmed parental relationships**
Phenotype highly specific for gene	2	1
Phenotype consistent with gene but not highly specific	1	0.5
Phenotype consistent with gene but not highly specific and high genetic heterogeneity**	0.5	0.25
Phenotype not consistent with gene	0	0

*Note that these points are *not* equivalent to the points used to classify a variant per the Tavtigian et al 2020 "Fitting a naturally scaled point system to the ACMG/AMP variant classification guidelines"
**Maximum allowable value of 1 may contribute to overall score

Table 2. Recommendation for determining the appropriate ACMG/AMP evidence strength level for *de novo* occurrence(s)

Supporting (PS2_Supporting or PM6_Supporting)	**Moderate** (PS2_Moderate or PM6)	**Strong** (PS2 or PM6_Strong)	**Very Strong** (PS2_VeryStrong or PM6_VeryStrong)
0.5	1	2	4

For all uses of *de novo* criteria, the phenotype in the patient must be consistent with the gene/disease association as recommended in the ACMG/AMP guidelines. When the patient's phenotype is consistent with the gene/disease association but not highly specific, we recommend decreasing the points awarded. For example:

- A patient with early infantile epileptic encephalopathy and a *de novo SIK1* variant with confirmed parental relationships is awarded 1 point (as the patient's phenotype is consistent with the gene but not highly specific and the variant is *de novo with confirmed parental relationships*). If this patient is the only *de novo* occurrence for the variant, then a Moderate strength level (PS2_Moderate) is applied.
    - If two additional unrelated patients with early infantile epileptic encephalopathy and a *de novo SIK1* variant with confirmed parental relationships are identified, then the combined point value is 3 (as each patient is awarded 1 point). For these combined occurrences, a Strong strength level (PS2) is applied as the points reach the Strong threshold (2 points) but not the VeryStrong threshold (4 points).

- A patient with nonsyndromic intellectual disability and a *de novo ASH1L* variant is awarded 0.5 points (as the variant is *de novo* with confirmed parental relationships and patient's phenotype is consistent with the gene but not highly specific and there is significant evidence of genetic heterogeneity). If this patient is the only *de novo* occurrence for the variant, then a Supporting strength level (PS2_Supporting) is applied.
    - If a second patient with nonsyndromic intellectual disability and a *de novo ASH1L* variant with confirmed parental relationships is identified, then the combined point value is 1 (as each patient is awarded 0.5 points). For these combined occurrences, a Moderate strength level (PS2_Moderate) is applied.

- A patient with developmental delay but no other features of Cornelia de Lange syndrome and a *de novo NIPBL* variant with unconfirmed parental relationships is awarded zero points as this phenotype is not consistent with the gene/disease association. If this patient was the only *de novo* occurrence for the variant, then no *de novo* criteria are applied.

Additional considerations for applying *de novo* criteria based on inheritance:

- Conditions with X-linked inheritance: if the variant occurs *de novo* in an unaffected carrier mother, and family history is consistent - i.e., she has no affected brothers/other male relatives apart from her affected son(s) – *de novo* criteria may be applied despite the fact that she is unaffected.

- Autosomal recessive conditions: for a *de novo* occurrence in a gene associated with a condition inherited in an autosomal recessive pattern without an additional pathogenic/likely pathogenic variant identified, the strength of evidence should be decreased by one level.

- Mosaicism: for cases with apparent germline mosaicism (multiple affected siblings with both parents negative for the variant), parental relationships must be confirmed in order for *de novo* criteria to apply.